15 May 2018 Volume 14 Issue 5

The Column

the interactive e-publication for the global separation science industry

Cover Story

2 **Evaluating Marine Algae for Therapeutic Effects**
Snezana Agatonovic-Kustrin, a professor in pharmaceutical chemistry at Monash University, in Kuala Lumpur, Malaysia, spoke to *The Column* about the development of a method to evaluate antidiabetic and antioxidant activity in marine algae using high-performance thin-layer chromatography (HPTLC) direct bioautography

15 May 2018 Volume 14 Issue 5

The Column

the interactive e-publication for the global separation science industry

Into The Deep

Evaluating the therapeutic effects of marine algae

UBM

Cover Story

2 Evaluating Marine Algae for Therapeutic Effects
Snezana Agatonovic-Kustrin, a professor in pharmaceutical chemistry at Monash University, in Kuala Lumpur, Malaysia, spoke to *The Column* about the development of a method to evaluate antidiabetic and antioxidant activity in marine algae using high-performance thin-layer chromatography (HPTLC)-direct bioautography.

Features

13 Novel Methods Using Mass Spectrometry for Food Safety—From Contamination to Nutrition
Ashley Sage, Jianru Stahl-Zeng, and Philip Taylor, Sciex
This article highlights three events that necessitate new method development to meet various detection needs, ranging from the detection of pesticides such as fipronil and glyphosate, to the detection and quantification of fat-soluble vitamins.

20 The *LCGC* Blog: Problems and Ionic Liquid Solutions for Soil BTEX Analysis
Kevin Schug, Department of Chemistry and Biochemistry, The University of Texas (UT) at Arlington
Sources indicate the composition of soil ideal for growing plants to be 25% air, 25% water, 45% minerals, and 5% organic matter. That does not seem like a daunting makeup, but the reality is that the relative proportion of the constituents can vary dramatically.

23 Modern PFAS Analysis: New Analytical Options for Testing PFAS in Various Matrices
Allen Misa and David Kennedy, Phenomenex, Inc.
The role of liquid chromatography tandem mass spectrometry (LC–MS/MS) in the detection of per- and polyfuorinated alkyl substances (PFAS) in a variety of matrices is discussed.

Regulars

6 News
The latest news and news in brief

10 Incognito
Do You Work in an Amazing Laboratory?
Is it time to update your working practices?

32 Training Courses and Events

34 Staff

Photo Credit: Hiren Ranpara/Shutterstock.com

Evaluating Marine Algae for Therapeutic Effects

Snezana Agatonovic-Kustrin, a professor in pharmaceutical chemistry at Monash University, in Kuala Lumpur, Malaysia, spoke to *The Column* about the development of a method to evaluate antidiabetic and antioxidant activity in marine algae using high-performance thin-layer chromatography (HPTLC)-direct bioautography.

—Interview by Kate Mosford

Q. You recently developed a method using high-performance thin-layer chromatography (HPTLC)-direct bioautography to evaluate antidiabetic and antioxidant activity in marine algae (1). What are the possible benefits of brown and green algae for diabetes prevention and management?

A: There is great interest in functional foods because they have the potential to improve and maintain human health. Therefore, they also have the potential to provide many growth opportunities for the food industry. Functional foods contain components that are either not present or are present at lower concentrations in similar conventional foods consumed as part of a usual diet. There is an emerging trend to use functional foods for the prevention of cardiovascular disease and diabetes. As the most ancient members of the plant kingdom, marine algae are well known sources of bioactive compounds, with a range of different biological activities. Marine sources have received great attention in the search for natural bioactive compounds and as a source of functional foods. Marine algae is increasingly being consumed for functional benefits beyond the traditional considerations of nutrition and health benefits. Marine algae or seaweed is the secret ingredient to a healthy heart. It is well known that people from Okinawa, Japan, have the longest lifespan. It is also known that they consume the most seaweed of all the world's population (2).

There have only been a few studies previously investigating alpha amylase inhibitory activity in marine algae (3–6).

2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

In these studies, they applied the alpha amylase test on the whole sample without chromatographic separation, and hypothesized that polyphenolic-rich extracts are responsible for antidiabetic activity. In our study, we reported on the alpha amylase inhibitory activity of natural products separated from selected marine algae extracts (1). For this work, an efficient experimental protocol for a HPTLC-alpha amylase enzyme inhibition assay was developed. After chromatographic separation of extracts and application of the alpha amylase essay test, we established that terpenoids are actually responsible for this activity.

Q. Can you describe the principles behind high-performance thin-layer chromatography (HPTLC)?

A: Planar chromatography and, of course, thin-layer chromatography (TLC) have been traditionally used for the qualitative analysis of mixtures, to characterize and track components visually, or as an initial separation technique. With technical improvements in recent years, high-performance thin-layer chromatography (HPTLC) has emerged as the most advanced form of TLC. This technique involves automation of a number of different steps, an increase in plate resolution, and the ability to allow both qualitative and quantitative analysis.

HPTLC plates offer a higher speed of separation and better resolution (clearer sample separation) than TLC plates because of smaller stationary phase particle size and associated larger surface area. The use of HPTLC plates—in combination with automated sample applicators, development chambers that enable gradient elution, high-resolution cameras, and computing software—allows for more control over experimental conditions and better analytical capabilities.

Q. What is HPTLC-direct bioautography and what benefits does it offer the analyst?

A: Bioautography combines chromatographic separation with biochemical assays, making it an incredibly effective method for *in vitro* screening for potential drug leads in complex samples such as plant extracts. The technical improvements in instrumentation and automatization of HPTLC, combined with biochemical (enzymatic) derivatization and direct hyphenation with spectroscopic techniques, enables parallel bio-profiling, bio-detection, and characterization of biologically-active compounds in a sample.

Q. What were the main obstacles you encountered developing this method and how did you overcome them?

A: The main obstacle was to effectively optimize experimental conditions and accurately and quantitatively compare alpha amylase inhibitory activity in different extracts.

Q. What are the advantages of using HPTLC over existing methods?

A: HPTLC has the ability to run many samples in parallel on the same plate, uses small volumes of solvent, is cost-effective, provides rapid analysis, gives an instant visual result, and is very user-friendly compared to high performance liquid chromatography (HPLC) or gas chromatography (GC). Results can be presented as chromatograms (peaks) and also as plate images. HPTLC also offers the option of multiple detection methods, before and after specific derivatization on the same plate. In column chromatography, separated compounds are identified by their Rf value. In TLC chromatography, compounds are identified by their Rf value and by their colour or fluorescence under UV–vis light, UV 366 nm and UV 254 nm, before and after derivatization. An automated multiple development (AMD) procedure in HPTLC

allows gradient elution, which in many cases can significantly improve component separation. Planar layer chromatography provides further advantages, such as minimal sample preparation. Crude extracts can be applied on the plate (spotted or sprayed as bands) without losing components during extraction or sample pretreatment. This approach also offers parallel profiling of samples on the same plate. Different effect-directed (bio) assays can be run because mobile phase can be removed after plate development, enabling direct post-chromatographic assays to be performed without much effort. Thus, various microchemical (derivatization reagent), biochemical (enzymatic), and biological (cell-based) assays can be performed directly *in situ* on the chromatographic plate. On the other hand, HPLC provides higher separation, but operates with solvents that are often toxic to biological cells and to enzymes, making it more difficult or impossible for the tests previously discussed to be performed.

Q. Do you think there are any misconceptions surrounding HPTLC that prevent it being more commonly used?

A: Yes, there are many misconceptions about HPTLC and TLC. TLC and HPTLC are seen as being "not reproducible" and only as a good "separation tool" for "semiquantitative determinations". However, modern TLC and HPTLC have the ability to perform quantitative analysis and furthermore, offer the possibility to incubate enzymes and viable cells directly on to the plate and to run bioassays. Furthermore, they can also be coupled on-line with spectroscopic methods for detection and identification, as well as with other analytical and preparative separation techniques.

Q. What are you working on next?

A: My current interest is in investigating cardiovascular effects of selected marine algae. We are investigating the interaction of bioactive compounds from marine algae extracts, previously separated on chromatographic plate, with nitric/nitrous acid. In this work, a polar silica (silicon dioxide) stationary phase acts as a support to bring phenols close to nitrates by forming a ternary complex through hydrogen bonding so that they react together.

References

1. S. Agatonovic-Kustrin and D.W. Morton,

Journal of Chromatography A **1530**, 197–203 (2017).
2. D.C. Willcox *et al.*, *Journal of the American College of Nutrition* **28**(sup 4), 500S–516S (2009).
3. P.S. Unnikrishnan, K. Suthindhiran, and M.A. Jayasri, *Pharmacognosy Magazine* **11**(Suppl 4), S511–S515 (2015).
4. N. Payghami *et al.*, *Pharmacognosy Research* **7**(4), 314–321 (2015).
5. P.S. Unnikrishnan, K. Suthindhiran, and M.A. Jayasri, *Frontiers in Life Science* **8**(2), 148–159 (2015).
6. C. Lauritano and A. Ianora, *Marine Drugs* **14**(12), 220 (2016).

Snezana Agatonovic-Kustrin received her Ph.D. in pharmaceutical chemistry and drug analysis in 1993, her Master of Science degree in pharmaceutical chemistry in 1988, and her Bachelor of Science in pharmacy in 1984. She has over 30 years' experience and background as an academic in different pharmacy disciplines worldwide. She has taught in a wide range of pharmacy subject areas (both at bachelor and master degree levels). She has been a pioneer in chemometry and experimental design, artificial neural network modelling, computational pharmacokinetic studies, and quantitative structure–activity relationship (QSARs) models. Her current research interest is in natural products and food chemistry, marine drugs, HPTLC bioassay guided high-throughput screening, HPTLC effect-directed screening, drug quality control, and colloidal drug delivery systems.

E-mail: snezana.agatonovic@monash.edu
Website: https://www.monash.edu.my/pharmacy/about/academic-staff/professor-snezana

2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

News

Postnova Announces Viral Collaboration with University of Helsinki

Postnova Analytics (Landsberg am Lech, Germany) has announced a joint collaboration with the Department of Biosciences at the University of Helsinki in Finland, to use asymmetrical flow field-flow fractionation (AF4) for the purification of halophilic viruses.

Viruses that persist in extreme environments, such as high salinity or high temperatures, are of particular interest to researchers because of their unique adaptations for survival, and one of the main challenges involved in studying these viruses has been to identify an efficient separation technique—in particular, a separation technique that both purifies and maintains the infectious nature of the viruses.

A recent publication to come out of the collaboration details the technique developed to purify and study extremophilic viruses (1). Four viral morphotypes were successfully purified with high virus recoveries and purities comparable to those of multistep ultracentrifugation purification methods. The paper also explains how to use AF4 as a rapid monitoring tool for virus production in slowly growing host cells living in extreme conditions.

For more information, please visit **www.postnova.com**

Reference

1. K. Eskelin *et al.*, *Extremophiles* **21**, 1119 (2017).

Restek and Girl Scouts Team Up to Promote STEM

Restek (Bellefonte, Pennsylvania, USA) has partnered with the Girl Scouts in the Heart of Pennsylvania (GSHPA) to host three STEM expos with the aim of building basic science skills in girls.

Organized by the GSHPA, the events will be attended by more than 1500 Girl Scouts of a variety of ages and will feature dozens of activities provided by programme partners to bring science, technology, engineering, and mathematics skill-building opportunities directly to girls.

Two of the STEM expos have already taken place in East Stroudsburg University and Pennsylvania College of Technology in Williamsport, with a third planned to take place at Millersville University on 19 May 2018.

Restek brought along two demonstrations, one in which the girls created a miniature lava lamp by adding Alka-Seltzer to a 40 mL vial containing a mixture of coloured water and mineral oil, and another involved a gumball separation demo.

"It felt like a big hit and they were excited they could take their lava lamps home to show their friends and family," said Alexandria Pavkovich, R&D Chemist at Restek. "I take a great interest in getting girls into science. Some of them are coming from a smaller area and it's fun to broaden their experience and spark an interest in science."

"GSHPA's mission is to build girls of courage, confidence, and character who make the world a better place," said Ellen M. Kyzer, President and CEO of GSHPA. "We are successful because supporters like Restek invest in our girls and help create the next generation of successful female leaders."

Other exhibitors at the event included Pocono Environmental Education Center, Scranton/Wilkes-Barre RailRiders, Association for Women in Mathematics, Wildlands Conservancy, Lincoln Caverns, AccuWeather, and Da Vinci Science Center.

For more information, please visit **www.restek.com** or for more information about Girl Scouts in the Heart of Pennsylvania, please visit **www.gshpa.org**

Photo Credit: De Space Studio/Shutterstock.com

Peak Receives Royal Visit to Celebrate 20 years

Peak Scientific has given a guided tour of their manufacturing headquarters in Inchinnan, Scotland, to his Royal Highness The Duke of Gloucester to mark the company's 20th anniversary

The recipients of six Queen's awards for enterprise since 2004, Peak Scientific are well known to members of the royal family, having previously received a royal visit in 2013 from her Royal Highness The Princess Royal, who opened the Cherry Tree House—an extension of Peak Scientific's headquarters.

The Duke toured Peak's factory learning about the process of producing laboratory gas generators, and also met with the workforce to learn about the ethos of the company and its history.

"We were honoured to welcome The Duke of Gloucester to our headquarters in celebration of our 20th anniversary. To have our hard work of the past two decades recognized in this way was truly heart-warming, and will remain a proud moment in Peak Scientific's history," said Robin MacGeachy, Peak Scientific's CEO.

For more information, please visit: **www.peakscientific.com**

A Nontargeted Metabolomic Approach for Organic Food Fraud

Researchers from the University of Almería, in Almería, Spain, have developed a nontargeted metabolomic approach to differentiate organic and non-organic tomato crops using high performance liquid chromatography–high-resolution accurate mass spectrometry (HPLC–HRAMS) (1).

The organic food market has seen exponential growth in the most recent decade as information regarding mass agricultural practices has become more readily available. Critiques, both justified and unjustified, have led many to seek out alternative crops grown in a more traditional way without the use of pesticides or other modern advances. The reduced yields of these growing practices are often represented in the premium that consumers pay for the products, with many costing considerably more than conventional food. However, the lack of reliable chemical markers to discriminate between these two products makes this market particularly susceptible to a less modern trend, food fraud.

As the second most important vegetable crop in the world, next to potato, the tomato (*Solanum lycopersicum L.*) represents an ideal crop to study in this context. A worldwide harvest of over 162 million tons annually, the tomato has also seen a rapid rise in the amount of land dedicated to its production organically. In Spain, the EU's second largest producer, the total number of hectares dedicated to organic tomato production increased by 66.3% in 2014 alone.

Organically grown food is often tightly regulated and there are few instances where the focus has been on conventionally produced products being sold as organic. However, doubts remain as to whether this type of food fraud is indeed rare or whether the low level of incidences are because of insufficient capabilities to detect fraud on behalf of authorities. For these reasons researchers investigated the potential of technologies such as HRAMS to identify suitable markers for distinguishing organic and non-organic tomatoes. Researchers also sought to combine data from isotope-ratio mass spectrometry (IRMS) and LC–HRMS analysis by chemometric methods as a tool to get robust classification models to discriminate between different practices. Finally, the study also sought to perform pesticide resident analysis to evaluate the influence of synthetic pesticides present in the secondary metabolites.

Results indicated the tested methodology would be an appropriate approach for distinguishing between organic and conventional tomato production practices. They found that methanol was a better extraction solvent offering better performance than acetonitrile, and also tentatively identified six markers for organic tomato production. IRMS analysis was found to be unsuitable for sole analysis because the variation of organic matter and nutrients incorporated during the crop can modify the $\delta^{15}N$ threshold data. Seven pesticides were successfully detected in conventionally grown tomatoes whereas only one was found in organic crops. While MS profiling data proved useful in combination with $\delta^{15}N$ data for sample clustering according to farming production systems, the MS profiling data are limited to specific crops. Therefore, researchers recommended a continuous build-up of HRAMS databases with different varieties and geographical locations to ensure authenticity of organic production.—L.B.

Reference

1. M.J. Martínez Bueno *et al., J. Chromatogr. A* **1546**, 66–76 (2018).

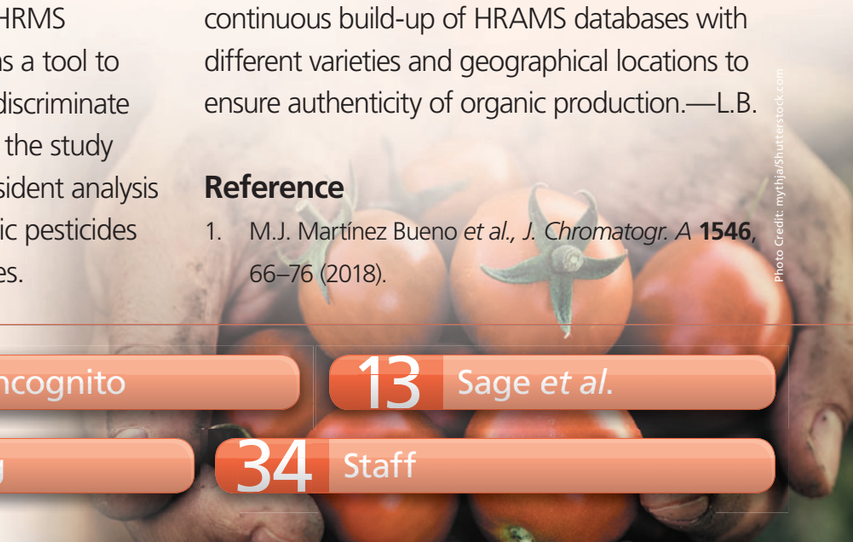


Photo Credit: mythja/Shutterstock.com

2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

Determination of Organometal Contaminants Using PTV GC–ICP-MS

Researchers from the Université de Pau et des Pays de l'Adour, in France, have developed a large volume injection method using a programmed temperature vaporization (PTV) injector for the simultaneous determination of mercury (Hg), tin (Sn), and lead (Pb) at ultra-trace levels in natural waters using gas chromatography–inductively coupled plasma mass spectrometry (GC–ICP-MS) (1).

Mercury, tin, and lead are among the most problematic organometallic species. Their high toxicity even at trace levels and ability to bioaccumulate and bioamplify along food chains makes them dangerous when they are present in the environment. Mercury in particular is widespread in aquatic ecosystems, and has both natural and anthropogenic origins as inorganic mercury (IHg) will form monomethylmercury (MMHg) through a biomethylation process, which will then bioamplify along food chains (2,3). MMHg is a potent neurotoxin (5), whereas the toxological role of IHg is still under discussion (6).

On the other hand, tin is a common contaminant in water environments because of its historic use in anti-fouling paints, pesticide formulations, wood preservatives, and polymer additives. As a powerful biocide, organotin compounds were regularly used on marine vessels until their ban in 2008 (6). However, their sedimentary legacy still remains, with dredging activities causing issues to resurface.

Similar to tin, lead contamination stems from human activity. The use of tetraethyllead as an anti-knocking additive in gasoline was a particularly common source until it was phased out in the mid-1970s (7). However, lead continues to find its way into the environment, from runoff waters from human activities such as mineral extraction and processing, smelting and refining, power generation, battery plants, and waste disposal or incineration (8). Organolead compounds are neurotoxic in nature (9).

With ever more stringent environmental quality standards (EQS) being set, the challenge for analytical chemists to detect trace levels of compounds in water environments has become greater. In order to monitor and investigate the fate of these compounds—and their many forms—in the environment, increasingly sensitive analytical methods are required. To address this, researchers sought to develop an on-line preconcentration method using a PTV inlet in combination with GC–ICP-MS to simultaneously determine the amount of Hg, Sn, and Pb in natural water.

The reported method was found to be very sensitive and, following optimization of the PTV parameters, absolute and methodological detection limits were found to be in the pg/L level, which is below EU requirements. Using unpolluted river water samples, researchers tested the applicability of the method with all targeted compounds being quantified with very good precisions.—L.B.

References

1. J. Terán-Baamonde *et al.*, *J. Chromatogr. A* **1547**, 77–85 (2018).
2. H. Hsu-Kim, K.H. Kucharzyk, T. Zhang, and M.A. Deshusses, *Enviro. Sci. Technol.* **47**, 2441–2456 (2013).
3. C.C. Gilmour, E.A. Henry, and R. Mitchell, *Enviro. Sci. Technol.* **26**, 2281–2287 (1992).
4. C.F. Harrington, *Trends Anal. Chem.* **19**, 167–179 (2000).
5. T.W. Clarkson, *Enviro. Health Perpect.* **110**, 11–23 (2002).
6. J.B. Graceli *et al.*, *Reprod. Toxicol.* **36**, 40–52 (2013).
7. UNEO 2015, Partnership for clean fuels and vehicles, http://www.unep.org/transport/new/pcfv/about.asp.
8. N.S. Duzgoren-Aydin, *Sci. Total Environ.* **385**, 182–195 (2007).
9. P. Craig, *Organometallic Compounds in the Environment* (John Wiley and Sons Ltd., West Sussex, UK, 2003).
10. J.Aszyk *et al.*, *J. Chromatogr. A* **1547**, 86–98 (2018).
11. B.K. Ambrose, B. Rostron, and N. Borek, *JAMA* **314**, 1871–1873 (2015).
12. S. Zhu *et al.*, *Tob. Control* **23**, iii3–iii9 (2014).
13. U.S Department of Health and Human Services, E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General (2016)
14. A. Khlystov and V. Samburova, *Environ. Sci. Technol.* **50**, 13080–13085 (2016).
15. C.A. Lerner *et al.*, *PLoS One* **10**, 1–26 (2015).
16. C.I. Vardavas *et al.*, *Tob. Induc. Dis.* **15**, 1–7 (2017).

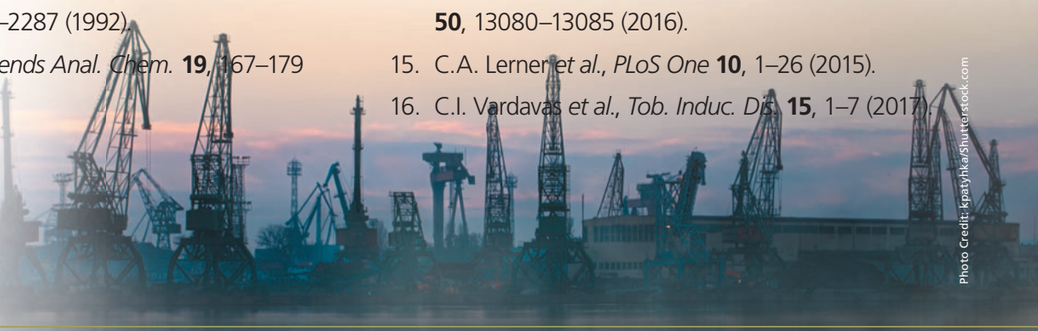


Photo Credit: kpatynka/Shutterstock.com

Peaks of the Month

- **The *LCGC* Blog: Do You Really Know Your Stationary-Phase Chemistry?**—What is the chemistry of this phase? What are the mechanisms of interaction with the analyte and hence how is retention and selectivity gained from this phase? How can we troubleshoot separation problems or develop suitable methods without a good knowledge of the bonded-phase chemistry? **Read Here>>**

- **A Compendium of GC Detection, Past and Present**—Gas chromatography makes use of a wide variety of detection methods. In addition to the most often used FID, ECD, TCD, and MSD, the list of other detection methods is long. They really shine when deployed properly, but their properties and applications can be a bewildering alphabet soup. This instalment presents a compendium of GC detection methods, both past and vanished as well as those that are current and relevant to today's separation challenges. **Read Here>>**

Credit: Cherdcha Chavivirojt/Shutterstock.com

- **The Mysteries of Marine Molluscs Revealed by Chromatography**—*The Column* spoke to Kirsten Benkendorff, an Associate Professor in Environment, Science, and Engineering and the Co-Deputy Director of the Marine Ecology Research Centre at Southern Cross University in Australia, about her work investigating Muricidae molluscs, their use in ceremonial incense and traditional medicines, and the role of chromatography in this research. **Read Here>>**

Credit: echo3805/Shutterstock.com

- **A Practical Approach to Modelling of Reversed-Phase Liquid Chromatographic Separations: Advantages, Principles, and Possible Pitfalls**—This article will describe, in a stepwise manner, how to perform successful and accurate retention modelling using reversed-phase LC examples and the pitfalls to be avoided to generate accurate predictions. The advice given is equally applicable to all types of retention models and applications using any of the commercial software programmes. **Read Here>>**

Credit: KucheAV/Shutterstock.com

- **Investigating the Role of Amino Acids in Celiac Disease Development**—Researchers from the University of Gothenburg, in Gothenburg, Sweden, have investigated whether plasma amino acid levels differed among children with celiac disease using LC–MS. **Read Here>>**

Like us Join us Follow Us

News In Brief

A European consortium consisting of groups from the Free University of Brussels, University of Liege, University of Ghent, and Katholieke Universiteit Leuven has won a 4 million Euro grant for work on liquid chromatography, gas chromatography, and chemometrics. Part of the Belgian government's drive to create centers of excellence in Belgium's leading research areas, the consortium will use the funding over the next four years on a project entitled "Chemical Data Mining in a Complex World". The project aims at the development of novel multi-separation methods and smart search and decision algorithms to make optimal use of the developed methods.

Eurofins Scientific has announced an agreement with LabCorp to purchase Covance Food Solutions, which provides product integrity, product safety, and consulting solutions for end-use segments that span the entire food supply chain. Covance Food Solutions operates an integrated network of 12 facilities across the world with nine in the US, two in the UK, and one in Asia. For more information, please visit: **www.eurofins.com**

Alexandra Knauer, of Knauer Wissenschaftliche Geräte GmbH, has been honoured with a VICTRESS Succession Award. The award ceremony honours outstanding women, who are role models and pursue visionary paths, and the VICTRESS succession award highlights a successful generational change in the management of a family business. "I am very happy about this award! I like to be a role model and inspiration for other women," said Alexandra. For more information, please visit **www.knauer.net**


Photo Credit: ASDF_MEDIA/shutterstock.com

Do You Work in an Amazing Laboratory?

Is it time to update your working practices?

I recently visited a laboratory where the working culture was different to many (perhaps any) that I've ever encountered. It made me reflect on the laboratories that I've worked in, and what makes a good working environment for the rather serious matter of high-quality analytical measurement.

The laboratory I visited struck me as different even before I entered; the sound of laughter coming from within as I donned the customarily ill-fitting visitor's lab coat in the vestibule area caught me off guard. Why should this be? Whilst I'm no important visitor, perhaps it's like the heads of state who think that everywhere smells of fresh paint, when visitors are expected, the analytical staff are told to "behave themselves". The laughter put me in a very positive frame of mind before the laboratory tour even began, and it really didn't lead me to think that the quality of the data produced would be any lower because of the jovial atmosphere.

It's true that we often need to concentrate to produce our best work, and that the health and safety implications of "larking around" in the laboratory are grave, but it was encouraging to hear a lighter hearted atmosphere in the laboratory; it really did instil an impression of togetherness and team work. I visit many working environments where the staff look cowed by the workload or the laboratory ethos. Analysts are under so much pressure to produce vast amounts of fit-for-purpose data, often without truly understanding the fundamental nature of what they are doing, and often feeling powerless to effect change. Perhaps they are bored with the constant routine of prepping samples, loading the autosampler, building the sequence, loading the method, pressing go, checking the QC or system suitability data, ending the run, and processing the data. So many never really get to the interesting aspects of the job: setting up the method on the instrument (rather than just loading it into the chromatography data system [CDS]), developing the methods, interpreting, reporting and presenting the data, and troubleshooting problems when things go wrong. A good working environment should encourage all staff to strive for more, to allow them to grow into areas of the science in which they are currently not engaged.

I find that thriving laboratory environments often encourage a sense of inquisitiveness and are happy for more junior staff to challenge the accepted norms. "Our guys are happy to follow the methods and get the job done" is not a statement that bodes well for scientific excellence, yet I hear it so often. "We don't pay then to think" or "We pay them from the neck down" are other damming statements that I've heard in the past, but which have been proven to be true. Truly, it doesn't have to be this way and I don't believe this is a good paradigm for high efficiency or good quality measurement. Senior staff should welcome and encourage challenge, and whilst not everyone will relish the opportunity to ask awkward questions, having a well-defined process in which to do so will lead to a much more satisfied staff. Whilst there will be limitations on what can and cannot be changed at the suggestion of more junior staff, understanding "why not" is much better than just being ordered to comply. A lack of openness to questions can be seen as failure to care or a shallowness of understanding on the part of senior staff, leading to a lack of confidence in the leadership, which unfortunately is often fully justified. The laboratory I visited had an electronic version of the suggestions box—an app in which non-urgent questions and suggestions were collected "on the fly" and which were thoroughly addressed in the open forum of a laboratory briefing on a weekly basis. I was shown some of the questions and they cut right to the heart of operational, technical, and organizational matters; there was no fear in the asking of a question and proper thought was given to the responses.

This laboratory also encouraged staff to undertake training of a meaningful nature every month. Yes, every month. It wasn't just a tick box on an annual staff review form, and neither was it crushingly expensive. The training often took the form of watching vendor videos and webcasts, speaking with more senior staff within the company (who were given time for such discussions), or wider reading and research on the analytical techniques that they were using. The difference was that the time for training was ring-fenced and was monitored to make sure that staff were truly given some thinking time each month.

Further, each staff member was given the opportunity to take on a special project from time to time, in order to break the inevitable monotony of the everyday analytical tasks. These projects are always focused on outcomes that will bring improvements to the quality, capability, or efficiency of the laboratory, and are therefore meaningful and rewarding. Access is granted to more senior staff who can be used as a resource to help fuel the progress of the project and, so I was told, the projects almost always result in some type of improvement. Again, these were monitored to ensure that everyone had the opportunity to shine.

The laboratory also had a very different approach to quality assurance and audit. Rather than being at loggerheads, the departments worked together to improve quality and the laboratory leadership encouraged openness and strove for high performance through a programme of recognition. I spoke to one member of staff who recounted their recent opportunity to present work at an international conference, which had been gained through working on a quality improvement initiative within the laboratory.

I spoke with several of the analytical team in the laboratory and asked each of them what they appreciated most about working there. The following list contains the most common themes that emerged:

- Budget and scope to introduce new technologies;
- Time to properly roll-out new equipment and software;
- High degree of automation for routine tasks;
- Ability to take work through, from sample booking in to reporting and explaining results.

I found it striking that whilst the general working ethos, hours of work, or pay were mentioned by some, the number of times the points above were mentioned indicate that challenge and investment in technology seem to have been more motivational to the staff.

The final note in my report to this laboratory simply says the words "help and support". All of the staff seemed to know where to go to for help on just about any problem. I asked about issues with scheduling, instrument problems, application issues, and software support and was always met with an assured answer on who they could seek guidance from and who their "expert" was on each of these subjects. I asked why there was such a great network of technique owners, subject matter experts, and fonts of knowledge and always got the same answer: the presence of a scientific leadership ladder within the business. There was the real possibility to progress in pay grade and seniority without having to leave the laboratory and be a desk jockey, which created a route of progression, filled with folks whose knowledge and skills had been retained within the laboratory (and indeed the business) and who could now be relied upon to teach and mentor the next generation.

For those of you thinking that this highly idealistic situation could never be achieved in a regulated modern laboratory environment, this was a contract research laboratory working mainly for pharmaceutical clients, performing product release and stability testing as well as less regulated work, including large-scale preparative chromatography.

For those managers wondering how there could be enough time for training and development projects, the answer is simple: time was made. The leadership had looked up from the grindstone and introduced a new paradigm, which had brought great success to the department and the business as a whole. They had been given no extra budget other than that required to retain scientific staff on management pay grades.

I know that this seems highly idealistic, but believe me, it's not fictitious and the really great thing was that for every job that came through the door, there were a handful of analytical staff who were clamouring to pick up the work. So, the question remains: if they can do it, why can't the rest of us attain this level of performance and motivation? Try to answer this question without using the words budget or time!

Contact author: **Incognito**
E-mail: **kate.mosford@ubm.com**

Novel Methods Using Mass Spectrometry for Food Safety—From Contamination to Nutrition

Ashley Sage, Jianru Stahl-Zeng, and Philip Taylor, Sciex, Framingham, USA

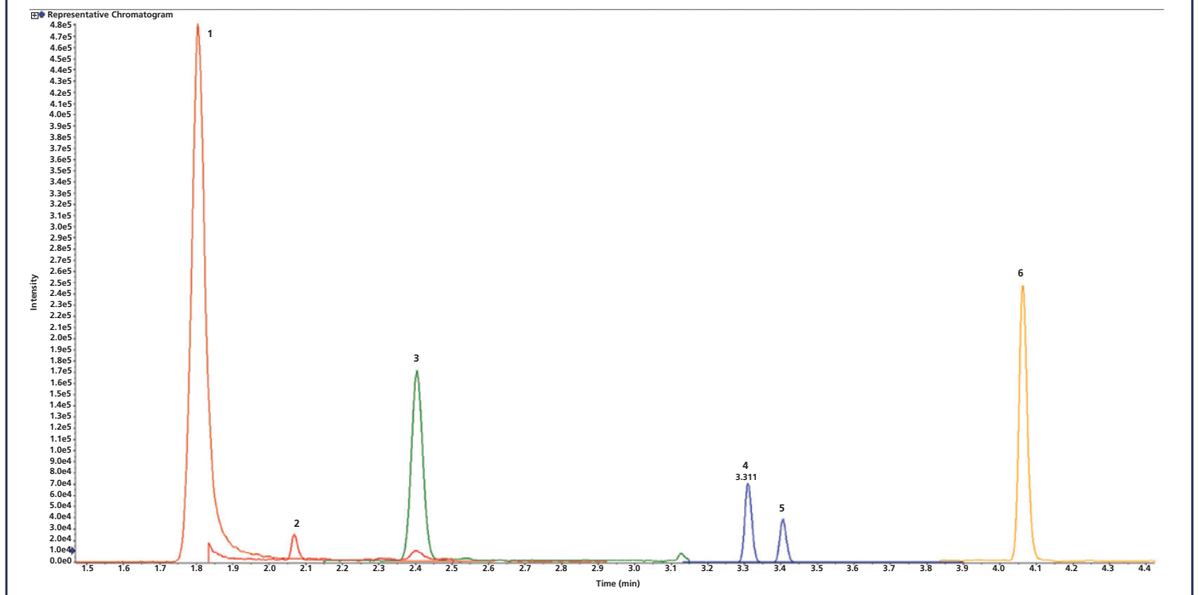
Modern eating habits have led to the further diversification of an already complex food supply chain. The public confidence in the food supply is not only impacted by publicized crises involving contaminations, but also through the misreporting of nutritional information. Food analysis is integral to the whole supply chain, be it through a rapid response to a food crisis, such as the fipronil egg scandal, the continued monitoring of pesticides that could harm an unsuspecting public, or accurately reporting nutritional information to provide the information the public need to make an informed decision about the food they eat. Each event is fraught with difficulties, but by developing new methods of analysis, crises in the food industry can be avoided or their effects mitigated. This article highlights three events that require new method development to meet various detection needs, ranging from the detection of pesticides such as fipronil and glyphosate, to the detection and quantification of fat-soluble vitamins.

Photo Credit: Trong Nguyen/Shutterstock.com

Modern diets have resulted in unprecedented growth and diversification of the food supply chain. The necessity of a continual supply of food means crops are commonly being treated with pesticides that are essential to reduce the risk of failed harvests. These chemicals are toxic to both insects and humans, and as such the public must be protected from unsafe concentrations of pesticides. Regulations imposed by food standard agencies dictate pesticide maximum residue limits (MRLs), which ensure the food on supermarket shelves is safe for consumption. In addition to ensuring food is free from contaminants, it is also essential to provide the public with accurate nutritional information to help enable the healthy growth of the global population.

To meet these goals, emphasis is placed on food content analysis to ensure quality and consistency between batches. Characterization and detection techniques, such as mass spectrometry (MS), offer manufacturers and producers the ability to screen large quantities of samples in a timely fashion, while guaranteeing reliable, repeatable measurements. Approaches to analysis are generally twofold: proactive monitoring of essential nutrients and possible contaminants; and crisis response, where widespread contamination requires the rapid development and deployment of analytical methods and equipment.

Figure 1: A chromatogram showing the clear separation of analytes, identifying fipronil and amitraz (another insecticide) and their related metabolites. 1. DPMF, 2. DMA, 3. DMF, 4. Fipronil, 5. Fipronil Sulfone, 6. Amitraz.

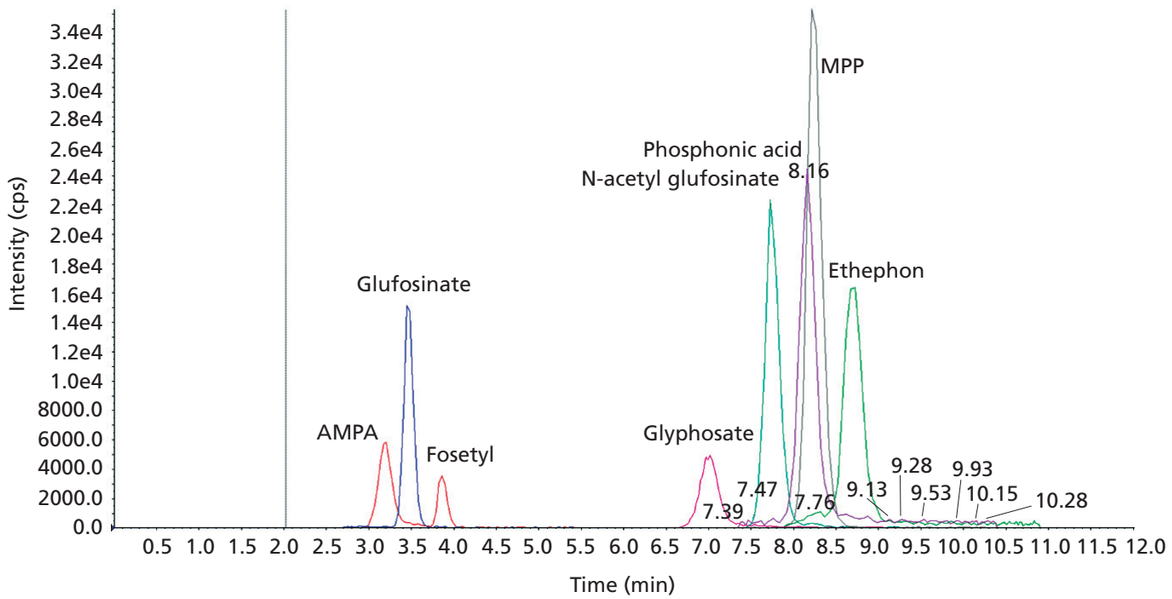


Rapid Response: Fipronil Egg Contamination

One recent example highlighting the need for a crisis response is the fipronil contamination of eggs, which resulted in the recall of millions of eggs. Fipronil is an insecticide, belonging to the phenylpyrazole family of chemicals, developed in the 1980s (1). Its high toxicity makes fipronil useful for controlling levels of insects including fleas, mites, and cockroaches, and is even useful against pests resistant to various insecticides (2). However, in July 2017 fipronil made news headlines after it was found to be present in eggs across Europe. This crisis was so widespread that by the end of August, fipronil-contaminated eggs were detected in 15 European countries and as far afield as Hong Kong and China.

Fipronil treatment for crops used in the food chain is prohibited and the US Environmental Protection Agency (EPA) has labelled fipronil as possibly carcinogenic,

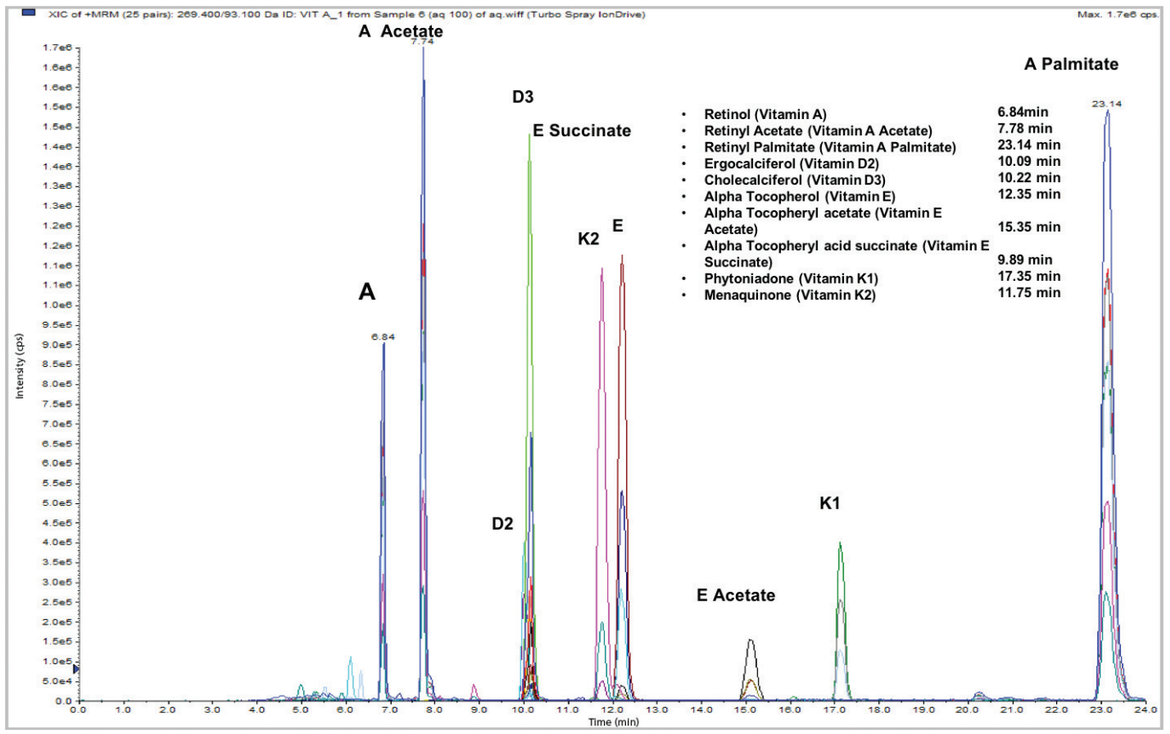
Figure 2: MS spectra provide the ability to clearly identify and distinguish glyphosate and its metabolite AMPA and other pesticides such as glufosinate.



prohibiting its presence in the food chain (3). Furthermore, the European Food Safety Authority (EFSA) dictates fipronil concentrations of under 5 µg/kg to be safe for human consumption (4). Complying with concentrations dictated by regulation is therefore vital for manufacturers and producers to ensure the continued safety and confidence in food supplied to the public. Detection methods form one essential part of this process, identifying and quantifying potential contaminations prior to distribution in the food chain. There are several plausible methods of detecting contaminants such as fipronil in samples of food. The most common detection methods include liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography–mass spectrometry (GC–MS), with both requiring different methods of sample preparation prior to analysis.

The challenge arises in detecting fipronil concentrations at the low level stipulated

Figure 3: A single chromatogram identifying the various vitamins that can be identified within a single mass spec run.



by government regulation, but novel methods capable of detecting fipronil and its associated metabolite, fipronil sulfone, have been developed. Since the fipronil contamination scandal began, demand for a fast, sensitive detection method has only continued to grow. One such detection method involves a modified QuEChERS sample preparation technique prior to detection using a triple quadrupole instrument and electrospray ionization (ESI). The high sensitivity of detection and ability to analyze two compounds in the same run enable regulation-compliant detection in a timely manner. This developed method can detect fipronil, as well as its major metabolite fipronil sulfone, to the MRL level of 5 µg/kg, as demonstrated in Figure 1 (5).

In responding to food contamination crises, it is also prudent to develop methods that can screen for multiple

16
2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

contaminants at once, potentially preventing contamination from other, unexpected sources. Using nontargeted approaches, such as SWATH acquisition, contaminant detection is not limited to the chosen molecule, in this case enabling the analysis of fipronil and other contaminants such as pesticides and polyaromatic hydrocarbons (6). Combining instrumentation that provides linear, reproducible contaminant detection to regulation-specific concentrations, with novel sample preparation methods, is essential to avoid repeated events, and helps improve public confidence in the integrity of the food supply chain.

Continual Monitoring: Glyphosate Pesticide

While fipronil is known to be hazardous for health, regulatory advice on other pesticides such as glyphosate is conflicted. Glyphosate is a widely used broad-spectrum systemic herbicide and crop desiccant. While it has recently made headlines for its potentially hazardous nature to humans as a possible carcinogen, its impact on human health is contested and therefore, the use of glyphosate as a farming pesticide is still permitted (7). In cases where potentially harmful pesticides are being used, continual monitoring is required to ensure chemical concentrations in foods are safe. Glyphosate is used globally and has been detected at trace levels, along with related metabolites, in 45% of European topsoils (8), and in samples of milk (9).

The controversy surrounding the use and potential contamination of glyphosate has placed greater emphasis on data collection and analysis methods to ensure levels of glyphosate in food samples fall below the safe MRL (reported by the EFSA as 50 µg/kg) (10). However, while the analysis of glyphosate and its associated metabolites is essential, its detection presents different sample preparation and analysis challenges that must be overcome. The high polarity of glyphosate and its related metabolites previously made sample extraction and LC analysis difficult. To overcome retention issues, derivatization using a method using fluorenylmethyloxycarbonyl chloride (FMOC-Cl) as the derivitization reagent to convert glyphosate into an analogue that can then be analyzed. However, while this approach enables detection, it is both complicated and time-consuming and fails to detect pure glyphosate and its metabolites.

Currently, the detection of underivatized glyphosate can be achieved using new methods of extraction coupled with

17
2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

instrumentation. One such underivatized method of detection starts by using the QuPPe (Quick Pesticide Preparation) extraction method to prepare samples (11). Using a combination of this approach with sensitive MS instruments, accurate quantification of glyphosate and its metabolites can be achieved. By combining the LC–MS/MS method with differential mobility separation (DMS) technology, interferences can be removed from analyses to improve the signal-to-noise ratio and, consequently, increase confidence in quantification results. These samples are then analyzed using LC–DMS–MS/MS to quantify and identify those contaminants present, as shown in Figure 2.

Vitamin Detection in Food

General food composition monitoring is not limited to the detection of harmful contaminants. It also forms an essential component of the accurate reporting of nutritional information for packaging labels. Vitamins are vital nutrients that are essential for an individual's growth and development. Deficiency in any vitamin is detrimental to health and is linked to a multitude of health issues. For instance, deficiency in vitamin D leads to the bone disorder known as rickets, and has been associated with other health problems including heart disease and cancer (12). While most vitamins can be obtained through natural means—exposure to sunlight is the best source of vitamin D—this is not always possible for some. Vitamin supplements are one option and are commonly incorporated into food, for example, infant formula. It is therefore imperative to report accurate nutritional information on food packages to ensure an individual's vitamin needs are met and for manufacturers to correctly advertise the benefits of their products.

Vitamins broadly separate into two categories: water-soluble (vitamins B and C) and fat-soluble (vitamins A, D, E, and K). Detection of water-soluble vitamins is relatively easy, with analysis possible using MS. Conversely, analysis of fat-soluble vitamins is difficult, owing to the challenges associated with MS detection. These problems originate from the presence of lipids in fat-soluble vitamin samples that cause an effect known as ion suppression (13), negatively affecting the detection, precision, and capability of a mass spectrometer. The ease in the detection of water-soluble vitamin samples is a direct result of the absence of lipids, enabling clean detection of vitamin B and C in samples (14). No uniform solution to ion-suppression exists, but its effects can be circumvented by the removal of lipids.

The detection of vitamins provides a specific detection challenge and, until recently, it was difficult to detect fat-soluble vitamins using LC–MS methods. Food samples contain various concentrations of vitamins, ranging from parts per billion to parts per million. To obtain clean, analyzable detection of fat-soluble vitamins, the lipid content should be removed from food samples. This reduces the ion suppression and enables robust MS analysis within a single chromatogram that identifies multiple vitamins, see Figure 3. Combining sensitive MS methods with associated expertise methodology allows universal application of the sample preparation and a simple analysis of vitamin concentrations in various food samples, meeting the specific requirements of the customer.

In Summary

Identification and quantification of potential contaminants and nutrients are essential to maintaining the integrity of the food supply chain. By avoiding unnecessary contamination of pesticides and providing clear labelling information, food manufacturers can be confident that food is safe for public consumption whilst also delivering accurate nutritional information. Continual methodological and instrumentation development will enable more sensitive and timely detection to continue for years to come.

References

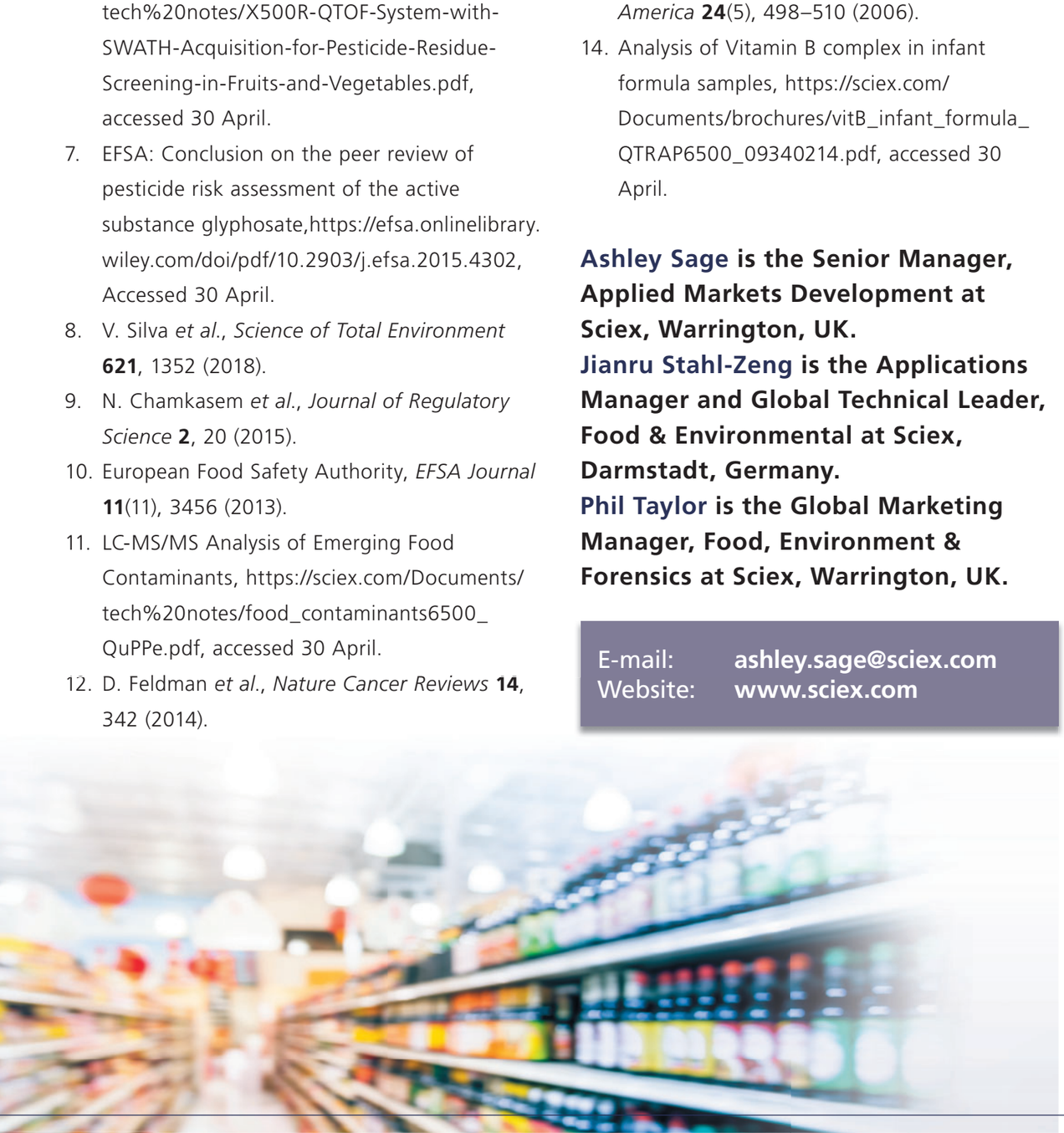
1. C. Tingle *et al.*, *Rev. Environ. Contam. Toxicol.* **176**, 1 (2003).
2. H. Kidd and D.R. James, Eds., *The Agrochemicals Handbook* (Royal Society of Chemistry, Information Services, Cambridge, UK, 3rd ed.,1991).
3. U.S. Environmental Protection Agency, *New Pesticide Fact Sheet - Fipronil; EPA 737-F-96-005* (U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office Washington, DC, USA, 1996), pp 1–10.
4. European Commission Pesticide Database, ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN, Accessed 30 April.
5. Analysis of fipronil and amitraz insecticides, sciex.com/Documents/tech%20notes/Rapid-LC-MS-MS-Method-for-the-Analysis-of-Fipronil-and-Amitraz-Insecticides.pdf, accessed 30 April.
6. Screening residual pesticides in fruit and

vegetables, https://sciex.com/Documents/tech%20notes/X500R-QTOF-System-with-SWATH-Acquisition-for-Pesticide-Residue-Screening-in-Fruits-and-Vegetables.pdf, accessed 30 April.

7. EFSA: Conclusion on the peer review of pesticide risk assessment of the active substance glyphosate,https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.4302, Accessed 30 April.
8. V. Silva *et al.*, *Science of Total Environment* **621**, 1352 (2018).
9. N. Chamkasem *et al.*, *Journal of Regulatory Science* **2**, 20 (2015).
10. European Food Safety Authority, *EFSA Journal* **11**(11), 3456 (2013).
11. LC-MS/MS Analysis of Emerging Food Contaminants, https://sciex.com/Documents/tech%20notes/food_contaminants6500_QuPPe.pdf, accessed 30 April.
12. D. Feldman *et al.*, *Nature Cancer Reviews* **14**, 342 (2014).
13. D. Volmer and L. Lee Jessome, *LCGC North America* **24**(5), 498–510 (2006).
14. Analysis of Vitamin B complex in infant formula samples, https://sciex.com/Documents/brochures/vitB_infant_formula_QTRAP6500_09340214.pdf, accessed 30 April.

Ashley Sage is the Senior Manager, Applied Markets Development at Sciex, Warrington, UK.
Jianru Stahl-Zeng is the Applications Manager and Global Technical Leader, Food & Environmental at Sciex, Darmstadt, Germany.
Phil Taylor is the Global Marketing Manager, Food, Environment & Forensics at Sciex, Warrington, UK.

E-mail: ashley.sage@sciex.com
Website: www.sciex.com



2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

The *LCGC* Blog: Problems and Ionic Liquid Solutions for Soil BTEX Analysis

Kevin A. Schug, Department of Chemistry and Biochemistry, The University of Texas (UT) at Arlington, Arlington, USA

I do not remember the application, but I remember very clearly Professor McNair telling us that soil is one of the most challenging sample matrices, if not the toughest, from which to perform analytical determinations. Sources indicate the composition of soil ideal for growing plants to be 25% air, 25% water, 45% minerals, and 5% organic matter. That does not seem like a daunting makeup, but the reality is that the relative proportion of the constituents can vary dramatically.

Photo Credit: domnitsky/Shutterstock.com

I have heard talks by Professor Milan Hutta about the complexity of humic substances (a major portion of the organic matter) and how soil is a model system for demonstrating the need for multiple and multidimensional separation approaches. Organic substances are present in a wide range of relative abundances and molecular weights; usually, we are interested in measuring some trace contaminant present in that matrix. Within the mineral portion are defined characteristics of the soil based on particle size (sand, 0.05–2 mm; silt, 0.002–0.05 mm; clay, <0.002 mm). These particles, which can also be mixed in any possible proportion, have different sorption characteristics. While sand and silt are largely inert pieces of rock, clay is largely made up from phyllosilicates, which are formed from the breakdown of other minerals. The amount of air and water in the soil will obviously vary with season and weather, and can even change daily. Spatially, in an area desired to be sampled, the composition of soil can even vary dramatically over short distances. These variations paint a picture of a highly variable matrix, which can contain virtually anything that has interacted with the environment.

We began our interest in soil contamination as part of the work we have been doing at the Collaborative Laboratories for Environmental Analysis and Remediation (CLEAR) at the University of Texas at Arlington. In an area of active unconventional oil and gas extraction in the Eagle Ford shale, we used a mobile mass spectrometer to measure benzene, toluene, ethyl benzene, and xylene (BTEX) released from gas flares on well-pad sites (1). In that work, we were able to pinpoint some inefficiencies in some of the well-pad

infrastructure designed to scrub BTEX from the waste stream.

This work also prompted us to measure BTEX in the surrounding soil (2). We found large amounts of BTEX, which had clearly been released from the gas flares and was deposited in the surrounding soil area. We used EPA Method 5021A (3) to carry out the soil analysis. This method uses headspace gas chromatography (GC) to liberate volatile organic compounds for analysis. However, to make an accurate analysis, you have to determine and match the soil composition of the real sample to build a calibration curve. This is an arduous task, where, before the headspace analysis of samples can commence, some of the soil sample is mixed with a soapy solution and allowed to settle into different layers, which can be used to estimate the amount of sand, silt, and clay present. The matrix can then be effectively mimicked using commercially available clean soils. Water is added to the soil mixture in the headspace vial to help normalize water content and liberate BTEX (or other volatile organic compounds) from the matrix. The biggest problem is that each soil composition can yield slightly different sensitivity (that is, the slope of the calibration curve can change drastically from soil to soil). Thus, without an appropriate surrogate matrix, it is very difficult to obtain reliable data.

Given recent development of the use of ionic liquids (ILs) as headspace cosolvents by Professor Armstrong and coworkers for the determination of water in various matrices (4,5), we decided to try ionic liquids as headspace cosolvents for BTEX determination. ILs are molecular molten salts, which can be tuned to exhibit a variety of physicochemical properties. They exhibit nominal vapour pressure and thus do not contribute to the analytes liberated into the headspace. For water analysis, it was important to choose highly hydrophobic ionic liquids, especially characterized by negligible water content. For BTEX, we chose to use highly hydrophilic ionic liquids—the idea is to homogenize the matrix and provide an environment that facilitates release of the BTEX into the headspace upon heating.

We recently published this alternate method (6). Importantly, using hydrophilic IL cosolvents, such as 1-ethyl-3-methylimidazolium ethyl sulfate ([EMIM][ESO_4]), 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][DEP]), and tris(2-hydroxyethyl) methylammonium methylsulfate ([MTEOA][$MeOSO_3$]), it was possible to normalize matrix effects for BTEX response from different soils. Additionally, because of the good thermal stability of the ILs, the temperature of the extraction could

be increased and equilibration time could be reduced (from 50 min to 30 min), relative to the EPA method. With such a method, it is no longer necessary to matrix-match blank soil samples for calibration. One calibration curve will suffice for analysis of BTEX from different soils.

The use of ILs as headspace solvents is not new (7), but the application to a challenging environmental soil matrix for BTEX determination is. Given the rise in industrial activity related to unconventional oil and gas extraction, as well as the down-stream industries that such activities facilitate (refining, specialty chemicals, and so forth), I think that more attention needs to be given to environmental BTEX exposure. These compounds—especially benzene—are hazardous to human health, and they can persist in the environment, given their chemical stability. There are an enormous number of routes that can bring humans into contact with BTEX. At least from the standpoint of soil, we have now afforded the ability to simplify BTEX determinations. It will be interesting to see other types of analysis for which this approach will become relevant.

References

1. Z.L. Hildenbrand, P.M. Mach, E.M. McBride, M.N. Dorreyatim, J.T. Taylor, D.D. Carlton Jr., J.M. Meik, B.E. Fontenot, K.C. Wright, K.A. Schug, and G.F. Verbeck, *Sci. Tot. Environ.* **573**, 382–388 (2016).
2. E. Varona-Torres, D.D. Carlton Jr., B. Payne, Z.L. Hildenbrand, and K.A. Schug, "The Characterization of BTEX in Variable Soil Compositions Near Unconventional Oil and Gas Development," in *Advances in Chemical Pollution, Environmental Management and Protection, Vol. 1: Environmental Issues Concerning Hydraulic Fracking*, Z.L. Hildenbrand and K.A. Schug, Eds. (Academic Press [Elsevier], Burlington, UK, 2017), pp. 321–351.
3. U.S. Environmental Protection Agency, Method 5021A. Volatile Organic Compounds in Various Sample Matrices using Equilibrium Headspace Analysis. Revision 2. July, 2014. https://www.epa.gov/sites/production/files/2015-12/documents/5021a.pdf
4. L.A. Frink, C.A. Weatherly, and D.W. Armstrong, *J. Pharm. Biomed. Anal.* **94**, 111–117 (2014).
5. L.A. Frink and D.W. Armstrong, *Food Chem.* **205**, 23–27 (2016).
6. E. Varona-Torres, D.D. Carlton Jr., Z.L. Hildenbrand, and K.A. Schug, *Anal. Chim. Acta*, in press. https://doi.org/10.1016/j.aca.2018.03.017
7. M. Andre, J. Loidl, G. Laus, H. Schottenberger, G. Bentivoglio, K. Wurst, and K.-H. Ongania, *Anal. Chem.* **77**, 702–705 (2005).

Kevin A. Schug is a Full Professor and Shimadzu Distinguished Professor of Analytical Chemistry in the Department of Chemistry and Biochemistry at The University of Texas (UT) at Arlington, USA. He joined the faculty at UT Arlington in 2005 after completing a Ph.D. in Chemistry at Virginia Tech under the direction of Prof. Harold M. McNair and a post-doctoral fellowship at the University of Vienna under Prof. Wolfgang Lindner. Research in the Schug group spans fundamental and applied areas of separation science and mass spectrometry. Schug was named the *LCGC* Emerging Leader in Chromatography in 2009, and most recently has been named the 2012 American Chemical Society Division of Analytical Chemistry Young Investigator in Separation Science awardee.

E-mail: **kschug@uta.edu**
Website: **www.chromatographyonline.com**

Photo Credit: antishock/Shutterstock.com

Modern PFAS Analysis: New Analytical Options for Testing PFAS in Various Matrices

Allen Misa and David Kennedy, Phenomenex, Inc., Torrance, California, USA

Per- and polyfuorinated alkyl substances (PFAS) are a rapidly growing environmental and human health concern. Owing to their broad commercial use, chemical stability, and bioaccumulation potential, these compounds are widely dispersed in the environment and can cause exposure through many potential pathways. To adequately estimate exposure risks, analytical methods are required that can measure low levels of PFAS compounds in many types of matrices. As will be described, recent advances in solid-phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC–MS/MS) have enabled the identification and quantification of a large number of PFAS compounds at low concentration (<1ng/g) in such diverse matrices as water, sediment, and dairy products.

What do people, polar bears, pizza boxes, and firefighting foams have in common? PBTs! Persistent bio-accumulative toxins (PBTs) have long been an environmental and health concern. Over the years, regulatory agencies have established guidelines and limits to protect the environment and human health from exposure to these compounds. Wel-known examples include: chlorinated dibenzodioxins (and related dioxin-like compounds), polynuclear aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs).

More recently, however, per- and polyfuorinated alkyl substances (PFAS) have become the next wave of PBTs to hit the global environmental testing radar.

These compounds have been widely used in commercial products as diverse as firefighting foams and pizza boxes. PFAS have been detected globally in the blood of mammals from cows to human beings and even in Arctic polar bears—very far removed from either aircraft fires or pizza boxes. As a result of these findings, in 2016 the U.S. EPA issued a drinking water health advisory level for PFAS at 70 parts per trillion. As more environmental monitoring data are collected, additional regulations are likely to follow (1,2).

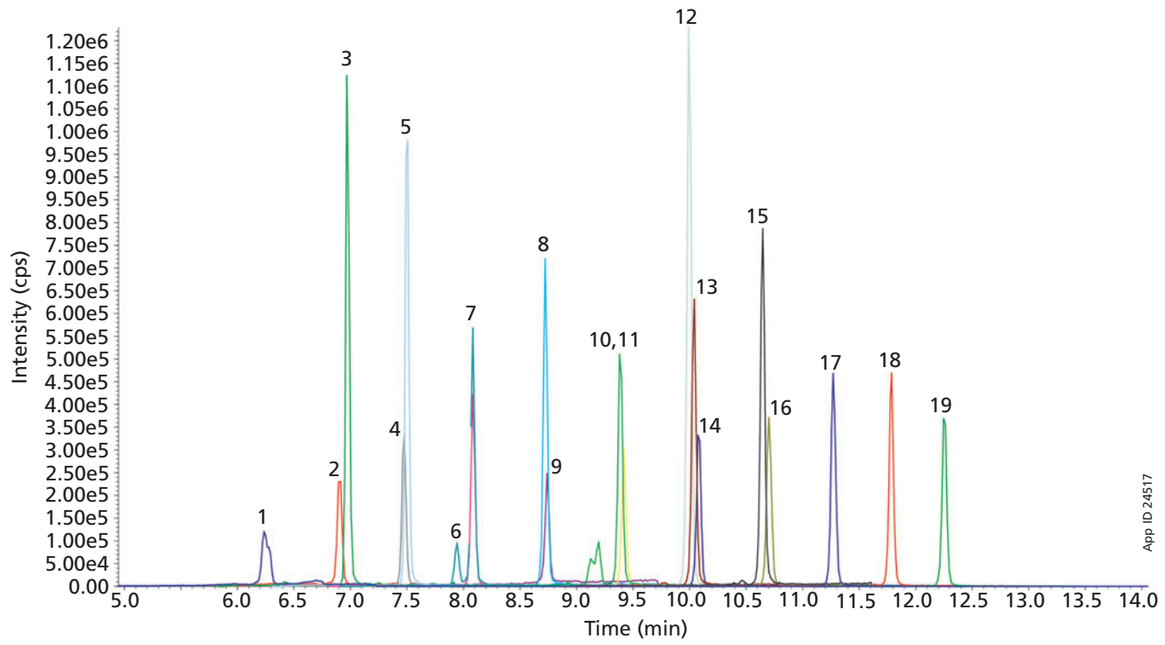
What are PFAS and Why is there Concern?

PFAS are long-chain aliphatic substances with fluorine substitutions on multiple carbon atoms (3). Owing to their thermal and chemical stability and their unique combination of hydrophilic and hydrophobic properties, PFAS have been used extensively in such diverse products as firefighting foams, non-stick food packaging, stain-resistant upholstery, and waterproof fabrics. They are very chemically and thermally stable and are resistant to degradation in aqueous environments, leading to trace-level contamination throughout much of the global water supply. As a result of their widespread use, environmental stability, and bioaccumulative propensity, some of the longer-chained PFAS compounds, such as perflurooctanoic acids (PFOA) and perflourooctane sulfonic acids (PFOS) have found their way into the human biome. A 2007 report found trace levels of PFAS in over 98% of the thousands of human blood samples tested (4). And, although PFAS are not acutely toxic, human exposure to PFAS residues has been linked to adverse health effects, perhaps owing to the endocrine-disrupting properties of these materials. As a result, analytical testing has greatly expanded beyond the initial focus on simple drinking water and is now being performed on diverse matrices such as soil, wastewater, and food. This expansion has revealed several new analytical challenges.

The Challenge of PFAS in the Analytical Laboratory Setting

PFAS analytical testing procedures generally use high performance liquid chromatography (HPLC) or ultrahigh-pressure LC (UHPLC) coupled with tandem mass spectrometry (MS) detection systems. As a result of the ubiquitous presence of PFAS in the environment, the contamination of laboratory blanks by trace levels of PFAS has become a great challenge. Potential PFAS interferences

Figure 1: PFAS analysis from sediment using QuEChERS and LC–MS/MS spiked at 1.0 ng/mL.



can arise from the analytical system components themselves, the laboratory water, or even "clean" analytical solvents. PFAS can be present in laboratory instruments and sample preparation and collection devices—particularly in plastic components—and can bleed into the flow path during analysis. Therefore, to ensure accurate results, the reduction of background PFAS contamination originating from both the preparation and LC systems must be a primary goal.

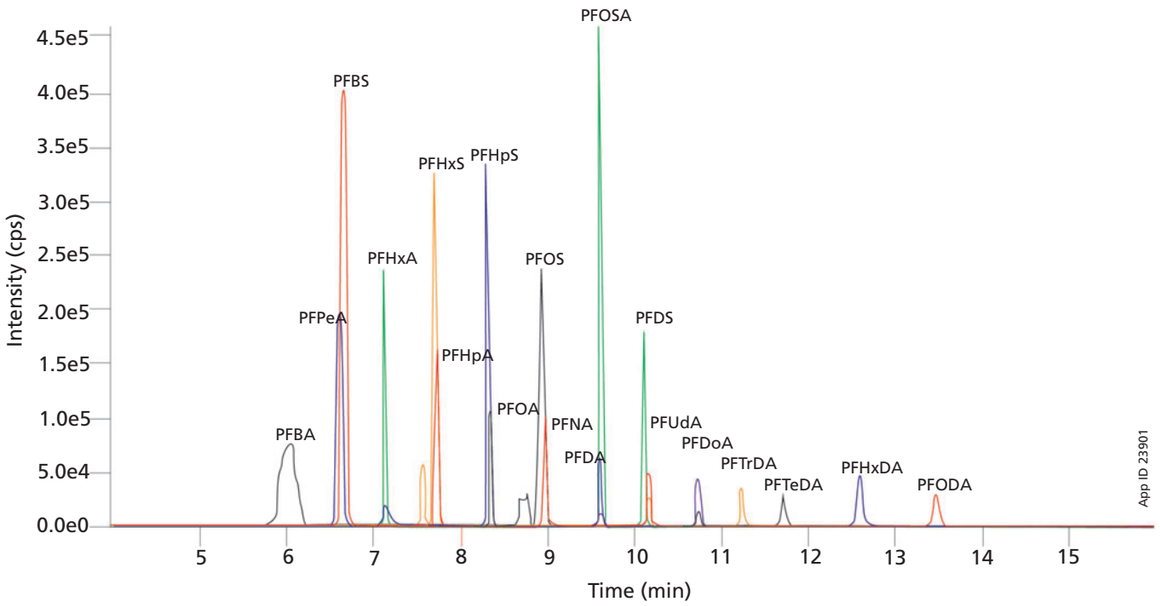
Techniques to Reduce PFAS Background in HPLC Analysis

A great deal of research has been done on background reduction. This has led to useful strategies for improved HPLC and UHPLC analysis for PFAS. Some simply remove or replace contaminated items from the workflow, others use specific products to separate system-generated PFAS from sample-related PFAS.

Some examples include:

Reduce system LC contamination:

Figure 2: PFAS direct inject with 10 ng/mL spike into water matrix diluted with methanol.



- Replace conventional tubing and filters with material free from fluorinated polymers
- Use vial caps without PTFE septa

Use a delay HPLC column to separate system PFAS from sample PFAS:

- Connect the delay column after the pump and before the HPLC column injector
- A short (50 mm) column length is usually sufficient; increase to 100 mm for higher levels of system PFAS contamination
- The delay column should be as retentive (or more retentive) than the analytical column, but efficiency and selectivity are not critical

In addition to these analytical system modifications, it is often beneficial to optimize the method for specific sample matrices. The use

Figure 3: PFAS in drinking water using online SPE and LC–MS/MS.

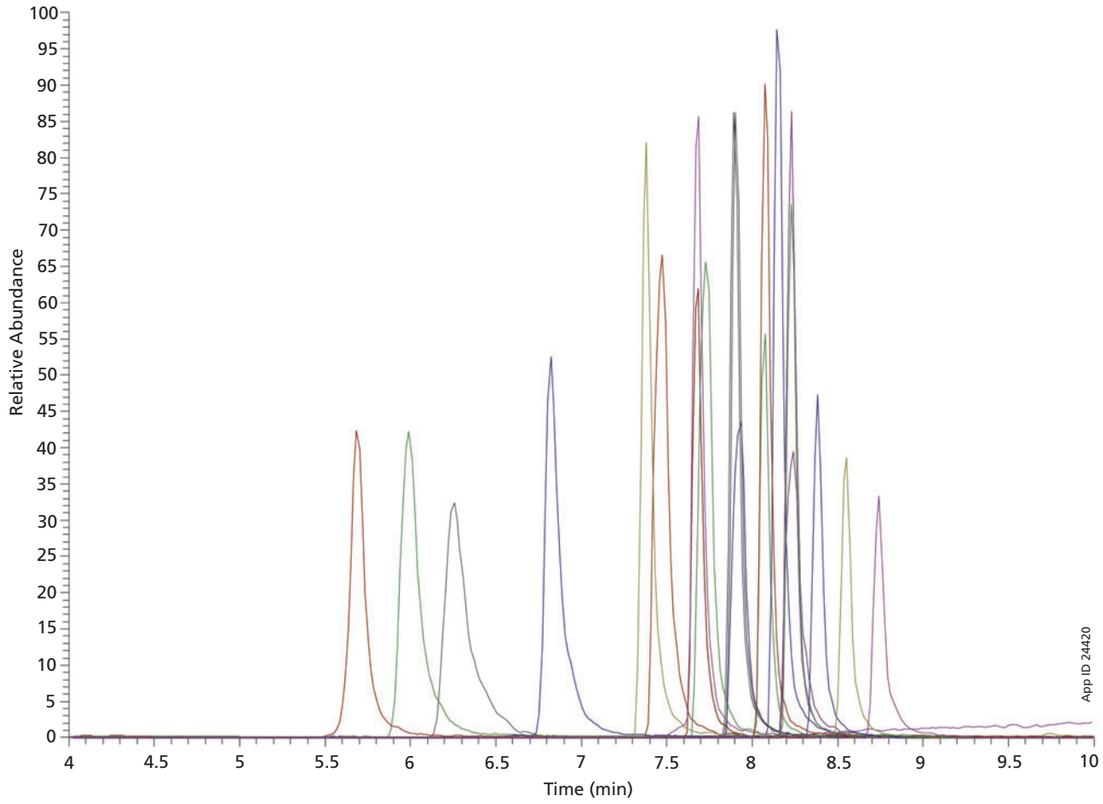
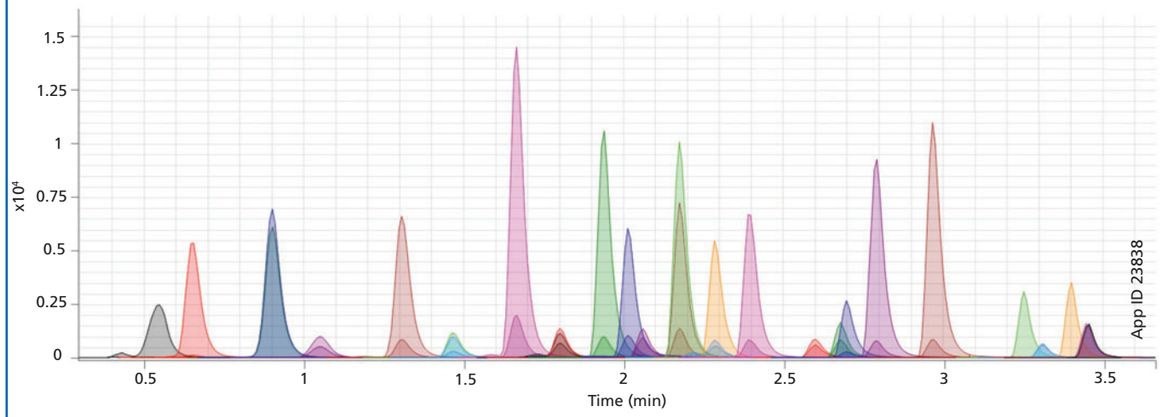


Figure 4: PFAS from foods by LC–MS/MS analysis at 0.05 ppb.



of QuEChERS, on-line and off-line solid-phase extraction (SPE), and direct injection often helps to improve method sensitivity while reducing accumulated PFAS background during sample concentration steps. The following examples will illustrate the wide array of options available.

Sample Preparation and HPLC or UHPLC Methods for PFAS Analysis in Environmental Samples

Determination of PFAS in Sediments Using QuEChERS Extraction and LC–MS/MS: Owing to the early EPA focus on PFAS in drinking water, many methods have been presented on the extraction and analysis of PFAS in this very simple matrix. However, literature offers far fewer examples of PFAS analysis in more challenging matrices such as sediments and sludges. Recently, the Los Angeles County Sanitation District developed and validated a procedure for the analysis of PPCPs, pyrethoroids, and now PFAS from marine and freshwater sediments using QuEChERS extraction and LC–MS/MS analysis (Figure 1) (5,6). This application of QuEChERS for the environmental analysis of marine and fresh water sediments represents a novel use of this sample preparation technique, which was originally developed (and is now widely used) for the analysis of trace levels of pesticides in food products.

While QuEChERS as a sample preparation technique is widely used for the preparation of food samples in the analysis of pesticide residues, it is now beginning to be applied to traditional environmental samples. In Figure 1, QuEChERS was shown to be a fast, efficient, and cost-effective procedure for extracting 19 PFAS analytes and resulted in average recoveries within 80–120% with the RSD of all analytes falling below 10%. PFAS, once released into the aquatic environment, will partition between the water phase and sediment. Therefore, to understand the fate and transport of these compounds, it is essential to accurately measure both the solid and

liquid environmental fractions. The use of QuEChERS sample preparation to remove matrix interferences allows these important distinctions to be made.

Quantitation of PFAS in Drinking Water Using Direct Injection by LC–MS/MS: As previously noted, the elimination of background contamination is a major challenge in PFAS analysis. Ironically, sample preparation and concentration steps such as liquid extraction and SPE are often the prime sources of such background contamination. Therefore, it would be desirable to devise analytical procedures that reduce or, ideally, eliminate sample preparation steps that involve contact with plastic components (7). In the case of a clean matrix such as drinking water, this goal is achievable through direct injection, provided that the LC–MS/MS instrumentation used is sufficiently sensitive to obviate the need for analyte preconcentration.

A direct injection method was developed by Test America in Sacramento, California, USA, for the quantitation of 17 PFAS analytes in water samples at the 1–10 ng/L level. The procedure featured the dilution of the analyte water sample in methanol and direct injection of 950 µL of the diluted sample into the LC–MS/MS system. However, to achieve such low quantitation levels, it was necessary to install a delay column to isolate PFAS contamination originating from the system pumps and eluents. A fully porous 5 µm 30 × 2.0 mm TMS-end-capped C18 HPLC column was installed outside of the column oven between the pump mixing chamber and the column for this purpose. A fully porous 3 µm 100 × 3.0 mm ethane-crosslinked C18 column was used for the analytical separation (Figure 2) (7). The application of a preliminary "delay column" ahead of the analytical column neatly circumvented the ubiquitous PFAS background interference arising from instrument and reagents that had plagued earlier PFAS methods (6).

Analysis of Perfluoronated Compounds (PFCs) including PFASs by On-line SPE and LC–MS/MS from Drinking Water: As mentioned above, off-line sample preparation techniques such as SPE can contribute to PFAS background contamination. However, on-line SPE has been shown to be an excellent way to avoid the off-line SPE contamination problem while still providing good analyte concentration and cleanup. On-line SPE is just as sensitive as its off-line counterpart (owing to larger injection volume onto the LC column), but it eliminates exposure to components used in off-line SPE that contribute to

PFAS background contamination. In addition, on-line SPE also significantly increases sample throughput and allows the collection of smaller sample volumes in the field. A method was developed and validated by Babcock Laboratories in Riverside, California, USA, using a weak ion exchange SPE column, sample preparation step, and a superficially porous, ethane-crosslinked C18 HPLC column. The weak anion exchange on-line SPE proved to be suitable for cleanup and concentration. It was also shown that the use of aqueous ammonia in the mobile phase, rather than the more commonly used ammonium acetate, provided a better MS-ionization environment for the 20 PFAS analytes. Furthermore, the use of a superficially porous, ethane crosslinked C18 column supported the higher pH contributed by the ammonia mobile phase, resulting in the overlaid chromatogram shown in Figure 3 (8).

What About Food?

Most environmental PFAS analyses discussed so far have been concerned with the traditional water, soil, and sediment phases that define pollutant disposal and transport. However, it is proven that pollutants that are discharged into the environment can potentially end up in the human and animal food supply. Owing to the widespread presence of PFAS in the environment and the high potential for bioaccumulation, this food safety concern is coming under much greater scrutiny. Even with the decreased use of PFAS in commercial products, this apprehension continues as a result of the exceptional persistence of PFAS in the environment.

Therefore, in addition to the increased frequency demand for PFAS analysis in traditional environmental samples, there is a growing interest in testing for low-level PFAS contamination in food matrices intended for human consumption. Described below is a fast and sensitive LC–MS/MS method codeveloped by Weck Laboratories and Phenomenex to analyze PFAS residues at low ppb levels in diverse food samples (Figure 4). High-fat matrices—milk, cheese, and fish—were intentionally chosen to challenge the method. Sample preparation is key to method performance; the traditional QuEChERS technique enabled 1 ng/g sensitivities, whereas an additional, optional SPE cleanup enabled 0.1 ng/g sensitivity and ensured optimum LC–MS/MS performance (9).

Conclusions

As a result of their unique chemical properties and widespread dispersion,

PFAS are now recognized as a much greater environmental challenge than originally imagined. As more information is developed, the scope and magnitude of the problem continues to increase. Consider how the initial focus on contaminated water has been followed by the knowledge of widespread presence in human and animal blood and has only now led to the investigation of potential transmission to the food supply. On the positive side—if there is a positive side—analytical science has now evolved to the state where a combination of new sample preparation techniques and advanced LC–MS/MS technology is widely available and capable of meeting the investigatory challenge. This tool set was not available even as recently as a decade ago.

Further Information

Readers are invited to visit **www.phenomenex.com/PFAS** where a snapshot of some of the best current PFAS analytical techniques has been compiled. Readers may also want to consider the 2018 National Environmental Monitoring Conference to be held **6–10 August 2018** in New Orleans, Louisiana, USA. Sponsored by the US Environmental Protection Agency, this conference will feature a full-day session titled "Characterization of Polyfluoroalkyl Substances in the Environment".

References

1. PFAS in Artic Birds: https://stud.epsilon.slu.se/6902/11/axelson_s_140625.pdf
2. PFAS in Polar bears: http://www.natureworldnews.com/articles/13479/20150316/pollution-giving-polar-bears-brain-damage.htm
3. PFAS in 98% of blood samples: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2072821
4. EPA 70 ppt Health Advisory: https://www.gpo.gov/fdsys/pkg/FR-2016-05-25/pdf/2016-12361.pdf
5. NEMC 2018: http://www.nemc.us/meeting/2018/confinfo.php
6. http://www.phenomenex.com/ViewDocument?id=determination+of+perfluoroalkyl+substances+(pfas)+in+sediments+by+quechers+and+lc-ms_ms+(tn-0117)
7. https://www.phenomenex.com/ViewDocument?id=analysis+of+perfluorinated+compounds+(pfcs)+in+aqueous+matrices%2C+evaluating+various+online+spe+sorbents+by+lc-ms_ms+(tn-0116)
8. https://az621941.vo.msecnd.net/documents/203693ce-3d2b-436b-abdd-3402d55f2b2b.pdf
9. Pierri, Agustin., Weck Laboratories, City of Industries, CA, USA

Allen Misa is the Global Marketing Manager-Food and Environmental at Phenomenex. He holds an M.Sc. in healthcare administration from California State University, Long Beach, USA, along with a B.Sc. in microbiology and chemistry from California State Polytechnic University, Pomona, USA. He is responsible for developing technical tools and resources for the use of chromatography in the food and environmental industries.

David C. Kennedy is Business Development Manager for Phenomenex. He is a graduate of Iowa State University with a B.S. degree in chemistry and a Ph.D. degree in analytical chemistry. His professional career has spanned over 45 years with a focus on food safety and environmental monitoring. He has had sequential assignments in industrial R&D, contract testing laboratories, and in the manufacture of analytical instrument and consumables.

E-mail: allenm@phenomenex.com / Davidk@phenomenex.com
Website: www.phenomenex.com

2 Q&A Agatonovic-Kustrin
6 News
10 Incognito
13 Sage *et al.*
20 The *LCGC* Blog
23 Misa and Kennedy
32 Events&Training
34 Staff

Training Courses

GC

The Theory of GC
Website: http://www.chromacademy.com/gc-training.html

GC Troubleshooting and Maintenance
Website: http://www.crawfordscientific.com/gc-troubleshooting-and-maintenance.htm

Hands-On Chromatography Training GC
Dates throughout the year
Chicago, Illinois,
USA
Website: www.ChromatographyTraining.com

Absolute Basics of GC and GC–MS
6 July 2018
The Open University,
Milton Keynes, UK
Website: https://www.anthias.co.uk/training-courses/AB-GC

HPLC/LC–MS

The Theory of HPLC
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/hplc-training.html

Fundamental LC–MS
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/mass-spec-training.html

HPLC Troubleshooter
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/hplc_troubleshooting.html

Hands-On Chromatography Training HPLC
Dates throughout the year
Chicago, Illinois,
USA
Website: www.ChromatographyTraining.com

HPLC Method Development
4 July 2018
London, UK
Website: www.hichrom.com

Hands-On Complete HPLC and LC–MS
23 July 2018
The Open University,
Milton Keynes, UK
Website: https://www.anthias.co.uk/training-courses/hands-on-complete-LC-LCMS

SAMPLE PREPARATION

Overview of Solid-Phase Extraction
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/sample-prep-training.html

GPC/SEC

Viscometry/Light Scattering/Triple Hands-On Training
21–22 June 2018
Mainz, Germany
Website: http://www.pss-polymer.com

MISCELLANEOUS

Basic Lab Skill Training
Website: http://www.chromacademy.com/basic-lab-skills-training.html

Introduction to IR Spectroscopy
Website: http://www.chromacademy.com/infrared-training.html

Introduction to Analytical Validation
Website: http://www.crawfordscientific.com/analytical-validation-training.htm

Separation of Biopolymers
29–30 October 2018
Victor's Residenz-Hotel,
Berlin, Germany
Website: www.molnar-institute.com

Users Training Meeting (UTM)
30 May–1 June 2018
Polymer Char Offices, Valencia, Spain
Website: http://polymerchar.com/users_training_meeting

Step-by-Step HILIC Method Development
11 July 2018
Cambridge, UK
Website: www.hichrom.com

Please send your event and training course information to Kate Mosford kate.mosford@ubm.com

Event News

3–7 June 2018

66th ASMS Conference on Mass Spectrometry and Allied Topics

San Diego Convention Center, San Diego, California, USA

E-mail: info@asms.org

Website: http://www.asms.org/conferences/annual-conference/annual-conference-homepage

29 July–2 August 2018

47th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2018)

Marriott Wardman Park, Washington, D.C., USA

E-mail: janet@barrconferences.com

Website: http://www.hplc2018.org

9–13 September 2018

1st International Conference on Ion Analysis (ICIA-2018)

Technische Universität Berlin, Berlin, Germany

E-mail: wolfgang.frenzel@tu-berlin.de

Website: www.icia-conference.net

17–19 October 2018

SFC 2018

Strasbourg, France

E-mail: register@greenchemistrygroup.org

Website: www.greenchemistrygroup.org

Contact Information

Europe

Vice President/Group Publisher
Michael J. Tessalone
michael.tessalone@ubm.com

Associate Publisher
Oliver Waters
oliver.waters@ubm.com

Sales Executive
Liz Mclean
liz.mclean@ubm.com

Sales Operations Executive
Kim Evans
kim.evans@ubm.com

Editor-in-Chief
Alasdair Matheson
alasdair.matheson@ubm.com

Managing Editor
Kate Mosford
kate.mosford@ubm.com

Associate Editor
Lewis Botcherby
lewis.botcherby@ubm.com

UBM Americas
Hinderton Point, Lloyd Drive,
Ellesmere Port, CH65 9HQ, UK
Tel: +44 (0)151 353 3621
Fax: +44 (0)151 353 3601

North America

Vice President/Group Publisher
Michael J. Tessalone
michael.tessalone@ubm.com

Publisher
Edward Fantuzzi
edward.fantuzzi@ubm.com

Sales Manager
Stephanie Shaffer
stephanie.shaffer@ubm.com

Sales Manager
Brianne Molnar
brianne.molnar@ubm.com

Editorial Director, Analytical Sciences
Laura Bush
laura.bush@ubm.com

Group Technical Editor
Stephen A. Brown
stephen.brown@ubm.com

Managing Editor
Megan L'Heureux
meg.l'heureux@ubm.com

Associate Editor
Cindy Delonas
cindy.delonas@ubm.com

Administation and Sales Offices
Woodbridge Corporate Plaza,
485 Route 1 South,
Building F, First floor, Iselin,
NJ 08830, USA
Tel: +1 732 596 0276
Fax: +1 732 225 0211

Corporate Office,
641 Lexington Ave., 8th Floor,
New York, NY 10022-4503, USA

Mission Statement
The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating, and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.

Custom Reprints: Contact Brian Kolb at Wright's Media, 2407 Timberloch Place, The Woodlands, TX 77380. Telephone: 877-652-5295 ext. 121. Email: bkolb@wrightsmedia.com.